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Novel roles for syndecan-1 in renal transplantation

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Chapter 7

General discussion and future perspectives

In this thesis we performed several studies with the aim to increase our understanding of the role of syndecan-1 in renal transplantation (RTx). Syndecan-1, a cell surface transmembrane HSPG, is well-known for its co-receptor functions for a variety of molecules including growth factors, adhesion molecules, cytokines, lipoproteins etc., thereby mediating a number of biological processes. Chronic RTx is often associated with extensive tissue remodeling, ongoing rejection, hypertension, dyslipidemia and proteinuria, which if not handled, eventually results in interstitial fibrosis/tubular atrophy (IF/TA) eventually leading to chronic transplant dysfunction (CTD) and graft loss. We investigated renal and hepatic syndecan-1 in a rat model of renal transplantation and also in experimental proteinuric conditions. In addition to the experimental studies, we also studied the role of syndecan-1 in human renal transplant recipients (RTR).

Syndecan-1 in renal tubular epithelial injury and repair

The role of syndecan-1 in epithelial regeneration and wound healing is well established (1-3). In chapter 2 we show that increased expression of tubular epithelial syndecan-1 in RTR is associated with improved renal functional performance and prolonged graft survival. The beneficial regenerative role of induced tubular syndecan-1 expression after renal injury was confirmed by both *in vitro* and *in vivo* studies. In chapter 3 we investigated shed syndecan-1 in plasma of RTR patients and its association with kidney function and endothelial dysfunction. In the same study we also showed increased tubular epithelial syndecan-1 expression in protocol biopsies taken one year after transplantation and its association with kidney function one, two and three years after transplantation. Together these data highlight the role of membrane bound syndecan-1 and soluble syndecan-1 in renal injury and repair after renal transplantation. Increased expression of syndecan-1 could be a response to kidney injury. In order to restore the damaged tissue, tubular cells dedifferentiate and enter a mesenchymal state in which they express syndecan-1. We propose that syndecan-1 acts as a co-receptor for various growth factors and integrin ligands, which might help in survival and re-epithelialization of the tubules (4). This idea is further supported by the association of tubular syndecan-1 with the proliferation marker Ki67 in renal biopsies, reflecting the role of syndecan-1 in restoring the early damage by proliferation and replacement of damaged cells.However, we speculate that when tubular damage outweighs the repair

mechanism as reflected by increased serum creatinine levels, the expression of syndecan-1 goes down by mRNA down-regulation and/or by shedding its protease sensitive ectodomain. The shedding is mediated by MMPs and results in unresponsiveness of the tubular epithelial cells to growth factors, which might lead to apoptosis and fibrosis. The concept of dedifferentiation of tubular cells upon injury, followed by re-differentiation into functional cells after repair or further dedifferentiation of the cells leading to fibrosis is well established (5).

The well-known renal biomarkers KIM1 (6), NGAL (7), CD44v3 (8), TLRs (9) are indicators of kidney damage and repair. Our results suggest that syndecan-1 might be a novel biomarker for repair and regeneration. The tubular syndecan-1 expression and its plasma concentration is representative of damage and ongoing repair possibly by binding growth factors such as HB-EGF (10), HGF (11), netrin-1 (12) thus promoting regeneration and repair. Thus more research is needed to validate syndecan-1 as marker of tubular regeneration and repair. Comparisons have to be made with the existing tubular injury markers mentioned above.

Shed syndecan-1 is found in plasma at low levels under physiological conditions as a part of regular turnover of syndecan-1. Increase in plasma syndecan-1 levels is reported under several pathological conditions. As mentioned earlier, in chapter 3 we report increase in plasma syndecan-1 levels in human RTR patients, which associates with endothelial dysfunction and kidney dysfunction. It would be interesting to know if this increase in plasma syndecan-1 is limited to renal transplantation or whether it could also be extended to native kidney diseases. Our group earlier showed increased tubular expression of syndecan-1 in IgA nephropathy and minimal change nephropathy where it could interact with L-selectin and IL-8 in tissue (13). We propose that an increase in tubular syndecan-1 is the result of tubular epithelial cells activation under proteinuric conditions. This induced syndecan-1 binds cytokines, which is then cleaved off from the surface together with the bound cytokine. This could be interpreted as a rescue mechanism to minimize the damage caused by these cytokines. Alternatively, also under proteinuric conditions epithelial syndecan-1 might be involved in tubular repair mechanisms.

Hepatic syndecan-1 and dyslipidemia in nephrotic syndrome:

A disturbed lipid profile is a common problem in renal patients, including transplant recipients. (14-16). Renal patients often suffer from unexplained dyslipidemia. Syndecan-1 is the primary HSPG mediating uptake of triglycerides in the liver (17,18). In chapter 4 we show increased expression and shedding of hepatic syndecan-1 and its association with dyslipidemia in an experimental rat model and in a human RTR cohort. The exact mechanism behind this increased expression is not studied but we show modification of hepatic HS side chains which in turn reduced their binding ability to lipoproteins. In chapter 5 we show similar findings in a rat model of spontaneous proteinuria. We demonstrate that caloric restriction not only restored blood pressure, proteinuria and kidney function to normal, but also improved lipid profile. The improvement in dyslipidemia is associated with reduced expression of hepatic syndecan-1 with increased sulfation of their HS side chains. These findings demonstrate that renal conditions affect hepatic syndecan-1/HS as a possible contributor to the associated dyslipidemia.

Renal conditions such as transplantation, renal ischemia, uremia and proteinuria, but also hypertension induce oxidative stress, which creates a pro-inflammatory state resulting in renal endothelial and epithelial cell activation. This, in turn, causes the systemic release of various pro-inflammatory cytokines and growth factors such as IL-6, FGF2, and TGF-β. It is quite possible that these circulating mediators trigger the activation of hepatocytes. Besides, proteinuria results in increased albumin synthesis in liver. It can be speculated that the liver specific protein synthesizing machinery is upregulated. Since syndecan-1 is abundantly present in the liver, its synthesis might therefore also increase under proteinuric conditions.

In chapter 4 we observed that despite pronounced syndecan-1 protein synthesis the TRL metabolism is hampered resulting in dyslipidemia. It is well documented that HS side chains of liver syndecan-1 are highly sulfated which is a prerequisite for binding and uptake of TRLs (19). In chapter 4 and 5 we show that there is modification of hepatic HS side chains, which hampers their binding to VLDLs. We also show increases in heparanase and MMPs which act upon syndecan-1 HS side chains and its ectodomain and cleave them off the cell surface thus blocking binding of TRLs, which interferes with TRL uptake by the liver. However, the actual mechanism leading to these changes under renal conditions is yet to be studied. It is crucial to understand how syndecan-1 and their sheddases, but also the enzymes involved in HS synthesis/sulfation are altered in

the dyslipidemia associated with renal conditions. This will help in designing interventions aimed at modulation of syndecan-1 and its HS changes, as a potential treatment strategy for the dyslipidemia in renal patients.

Matrix proteoglycans and renal fibrosis

In chapter 6 we showed up regulation of glomerular HSPG perlecan in renal allografts at both the mRNA and protein level. We also showed a change in binding properties of glomerular HS. Glomerular HS of renal allografts binds pro-fibrotic growth factor FGF-2 and contributes to its accumulation in glomeruli. Perlecan-positive mesangial cells proliferate in response to FGF2 in an HS-dependent manner. These findings clearly indicate the role of HS side chain modifications in accelerating the disease process. The pro-inflammatory milieu drives the modification of HS side chains to FGF-2 binding HS side chains. These changes can be a target of intervention to limit the fibrosis. These data show that besides the transmembrane HSPG syndecan-1, which in the kidney is primarily involved in proliferation and regeneration of tubular epithelial cells, matrix HSPGs such as perlecan are primarily involved in proliferation of mesangial cells and glomerulosclerosis. Apparently, proteoglycans serve as docking structures for GFs, and are thereby being involved in both regeneration and fibrosis. This is very context dependent and is mainly determined by the growth factors (being eg., FGF2 and TGF-beta to promote fibrosis and eg HB-EGF, netrin-1 and HGF to promote regeneration).But it is also cell-type dependent: proliferation of tubular epithelial cells is mostly related to regeneration (exception in polycystic kidney diseases), whereas proliferation of mesangial cells is related to glomerulosclerosis.

Future Perspectives:

Soluble syndecan-1: marker of tubular injury /regeneration?

As described earlier, the intact syndecan-1 ectodomain (together with HS chains) is cleaved and shed by the action of MMPs (20). The shed syndecan-1 is biologically active and retains its binding ability to various cytokines, growth factors, and lipoprotein. Increase in plasma syndecan-1 levels is observed under several pathological conditions such as trauma, bacterial infections, systemic inflammation, sepsis, and cancers (21-24).In chapter 3 and 4 we show that plasma syndecan-1 concentrations are increased in

renal transplant recipient patients. This increase is associated with renal dysfunction, endothelial dysfunction and dyslipidemia. Additionally we also measured plasma syndecan-1 in a small human proteinuric cohort (n=11) upon anti-proteinuric intervention with Indomethacin. We observed that 4 out of 11 patients, who had responded to the treatment, also show normalization of initially increased plasma syndecan-1. This suggests to us that perhaps syndecan-1 could be a potential marker of tubular injury or regeneration and probably its plasma levels are lowered upon recovery from injury.

It would therefore be interesting to measure syndecan-1 in paired plasma and urines samples of renal patients from larger renal cohorts from diabetic and proteinuric patients. Studying the relation of syndecan-1 with other tubular injury markers would be interesting as well, especially after effective therapeutic interventions. Since the shed syndecan-1 ectodomains are biologically active it is possible that shed syndecan-1in plasma/urine is bound to some potential cytokines (13), lipoproteins (25), inflammatory mediators or growth factors, which are abundant under pathological conditions. Thus, determining its binding partners could be of clinical relevance. This would also help us understand the sulfation dependent binding properties of HS side chains on syndecan-1 to various mediators under normal and pathological conditions.

Can hepatic syndecan-1 explain renal dysfunction-associated dyslipidemia?

It is fascinating to realize that renal abnormalities affect liver syndecan-1, which associates with dyslipidemia. It would be interesting to further explore whether the hepatic changes of syndecan-1 and HS side chains is limited to renal transplantation and proteinuria, or, alternatively, could be a general phenomenon in renal diseases. To gain more insight, cohort studies such as larger human renal cohorts with interventions targeting improvement in plasma triglycerides would be helpful. Plasma syndecan-1 values could potentially be associated with clinical outcome measures and lipid parameters, Assessment of such associations could help us determine whether or not hepatic syndecan-1and HS changes are involved in dyslipidemia in renal patients, as part of better understanding of the underlying causes of dyslipidemia in these patients. In chapter 5 we show that the caloric restriction has clear effect on syndecan-1, its HS side chain modification and also HS synthesizing enzymes such as sulfotransferases and HS degrading enzymes (sulf-2 and heparanase). To unravel the underlying mechanisms,

in vivo triglyceride clearance studies in rodent models, which specifically in their livers are knocked down for syndecan-1, and/or SULF2, and/or ADAM17 can be designed, with, for instance, injection of labelled VLDLs into these mice and assessment of their plasma clearance. Since these genes and their expression are altered in renal conditions it would be interesting to see whether knocking down these genes has any consequence on plasma triglyceride clearance and syndecan-1 expression and shedding. In addition, these models allow studying the impact of dyslipidemia on the progression of renal diseases. Experimental animal studies with pharmacological interventions targeting SULF2 and/or HS synthesizing enzymes can be designed to define the mechanistic role of these enzymes in regulating dyslipidemia via hepatic syndecan-1. However, this is very challenging, since specific inhibitors of HS synthesizing/degrading enzymes are barely developed yet, because the HS biosynthetic machinery is very complex and targeting a single enzyme might not result in desired modification. However, introducing HS analogs that bind to these enzymes as substrates and preventing them from exerting their action on endogenous HS side chains could be an option.

Dietary intervention studies such as caloric restriction in kidney diseases could improve kidney function and also lipid profile (26-28). Measuring plasma syndecan-1 in renal patients with caloric restriction/ dietary intervention before and after intervention and eventually associating plasma syndecan-1 concentration with lipid profile markers and kidney function markers can support the importance of syndecan-1 in dyslipidemia in these patients.

Additionally in vitro studies can be designed such as stimulating primary hepatocytes and / or renal tubular epithelial cells with serum from renal patients and healthy subjects (or the tubular cells with urinary proteins), to study the changes in cell morphology and behaviour and also quantitate the changes in syndecan-1, various HS synthesizing enzymes, sulfatases and matrix metalloproteases and signalling pathways involved. Such studies to some extent mimic the changes a cell usually would undergo in pathological condition and might help us understand the underlying mechanism and pave the way towards rational interventions.

Our work presented in chapter 2-5 of this thesis, together with the findings of others in different conditions (surgery, septic shock, liver diseases) suggest that increased plasma syndecan-1 could be a sensitive marker for tissue injury, and thus might be of clinical relevance. To determine whether plasma syndecan-1 levels have any diagnostic

importance in renal and other patients it is important to understand the origin of plasma syndecan-1, either from the diseased kidney or from the liver, or even from other cells/tissues such as endothelial glycocalyx or plasma cells. Experimental work in various animal models could shed light on this issue, which is currently a black box.

HSPGs as therapeutic targets:

In chapter 6 we show a role for perlecan-FGF2 in transplantation associated tissue remodelling and fibrosis. Intervention of exogenous heparinoids could be an option. These exogenously introduced heparinoids might competitively bind to perlecan and disrupt FGF2 binding with perlecan and retard fibrosis and matrix accumulation. However, this approach also has some disadvantages. These exogenously introduced heparinoids might also bind to growth factors involved in regeneration, and TRLs binding and uptake by the liver might be hampered as well. Hence this approach might need further tailoring. An intervention study has been performed by our group to minimize tissue remodeling and fibrosis in a rat model of renal transplantation (female Dark Agouti to male Wistar rats). Two chemically modified non-anticoagulant heparinoids, next to unfractionated heparin were injected sub-cutaneously into the rats after allo-transplantation. This intervention however did not result in significant attenuation in renal fibrosis. In order to achieve this goal we need to design/ synthesize highly specific oligosaccharides with defined narrow binding properties that would specifically bind to our target of interest, in this case FGF2. Alternatively, small peptides corresponding to the FGF2 domains binding to HS, but not able to activate FGF receptors could be given in order to prevent native FGF2 – HS interactions.

CONCLUSIONS

The data presented in this thesis reflect the role of syndecan-1 in renal condition. Both our *in-vitro* and *in-vivo* findings provide better insight in understanding the two potential roles of syndecan-1; firstly in renal tubular regeneration/repair and endothelial dysfunction post RTx. Secondly, the effect of renal conditions on hepatic syndecan-1 leading to dyslipidemia in renal patients. This data is of crucial importance in terms of designing intervention studies to limit tubular damage and dyslipidemia, which are among the major concerns in renal patients.

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